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Heavy metals concentration in oyster *Crassostrea sp. HZ*, sediment and sea water, Musa estuary

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Abstract

Heavy metals are permanent pollutants that are able to accumulate in the tissues of marine organisms. Toxicity and bioaccumulation of heavy metals in aquatic organisms have been considered as a critical problem. New species oyster *Crassostrea sp.HZ*, that live near petrochemical complex in the Musa estuary, especially may be exposed to considerable amounts of heavy metals from surrounded environment. The primary goal of this study investigates the concentration of Cd, Cu, Zn and Pb in recently reported oyster *Crassostrea sp.HZ* and its habitat in Musa estuary. Oyster, sediment and sea water samples were obtained from six selected stations. The soft tissue of oysters was separated. All of the samples were acid digested and their heavy metals level were analyzed using atomic absorption spectrometer. The concentration of Cd, Cu, Zn and Pb in soft tissues ranged 12.21-17.5, 207.2-403.52, 90.43-104.12 and 0.07-0.95 (µg/g), respectively. The concentrations of the same metals in sediment were 0.15-0.85, 1.99-141.42, 60.42-122.1 and 2- 7.2 (µg/g) respectively. The concentrations of heavy metal in sea water was ND- 0.7, ND-3.1, 9.12- 23.03 and 0.15-2.27 (µg/g), respectively. The result showed that the level of heavy metals in soft tissue of *C. sp.HZ* was higher than general standard.

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Introduction

Metals are natural elements which continually discharged into the environment by alterations of their geochemical cycles, either through human activities or natural processes including volcanic eruptions, soil erosion, mining and smelting activities, coal and petroleum combustion and etc. Besides, a variety of industries may also contribute to the contamination of the environment by metals. In marine environment, heavy metals appear in different forms including dissolved cations and metals bond to suspended organic materials (Al-Yousuf *et al.* 2000; Turkmen *et al.* 2005). All forms of heavy metals in aquatic ecosystems may be up taken by marine organisms and ultimately enter aquatic food chains and accumulate in various concentrations in organism's tissues (Tuzen. 2009; Altindag and Yig, 2005). Heavy metals are permanent pollutants that cannot be degraded for several years (de Mora *et al.*, 2004). The global concern on the presence of heavy metals in marine environments resides in their ability to be accumulated in the tissues of marine organisms. These elements eventually become toxic for organisms and human being upon consumption (Burger *et al.* 2005; Durrieu *et al*, 2005). In recent decades, toxicity and bioaccumulation of heavy metals in aquatic organisms have been considered as a critical problem (Usero *et al.*, 2005). Although, anthropogenic processes, in particular industries, are believed to be the main reason for increasing the amount of heavy metals in the environment, especially in aquatic ecosystems, however, this fact that environment itself has various amounts of heavy metals, cannot be denied.

The Musa estuary is a 60 Km length waterway system, which connected to the northwest Persian Gulf. This estuary ends into Mahshahr and Sarbandar cities, in southern Iran, and is divided into several branches in the cities. This estuary is situated close to the Emam Khomeini port that is one of the biggest ports in Iran. A wide range of point and non-point sources such as petrochemical industries, oil transportations and agriculture activities constantly discharge substantial quantities of anthropogenic contaminants into the estuary (Safahieh *et al*, 2011). Gathering data on heavy metals concentration in aquatic ecosystems without doubt is useful to estimate the level of heavy metals contamination in marine environment and its flora and fauna. Musa estuary as a special environmental ecosystem is important. It plays an important role in fisheries as capturing site and nursery ground for aquatic organisms. Therefore frequent monitoring in this area is necessary. Biomonitoring be conducted by endemic species in this region. Bivalves especially oysters have been studied frequently. *Crassostrea sp.HZ* oyster has recently been recorded in NCBI[1], *Crassostrea sp.HZ* oyster has recently been recorded in NCBI, especially in great abundance in various part of Mahshahr city. This study aimed to determine the level of heavy metals in *Crassostrea sp.HZ*, sediment and water from some branches of Musa estuary in order to valuate heavy metal pollution in the area.

Materials and methods

Sea water, sediment and oyster samples were collected from six sampling sites including Petrochemical quay (S1), Dock SorSoreh (S2), Khor-Zangi (S3), Quay 18 (S4), Quay 33 (S5) and Khor-Jafari (S6) (Fig. 1) during January of 2012. Thirty oysters, five sediment and sea water samples were obtained from each station. Surface water samples were collected by using polyethylene bottles (washed with nitric acid and then rinsed with double distil water), then they were acidified with 10% HNO_3 and filtered through a 0.45 µm membrane filter. The oysters and sediment samples were transferred to the laboratory, using icebox and were kept frozen at -20 °C until analysis.

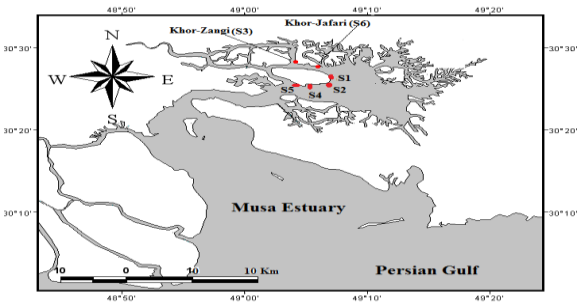

Fig. 1. Sampling stations in the Musa estuary.

Before analysis, the samples were thawed at room temperature. Each oyster sample was dissected for its soft tissue using stainless steel scalpels. The soft tissue of 5-6 oysters was pooled together to obtain sufficient weight for analysis. The soft tissues were freez-dried until reached a constant weight. About 1g of dried tissue predigested first at 80 °C for 1h and digested in 140 °C for another 3h with concentrated nitric acid until the soft tissue were completely disappeared. Sediment samples were freez-dried until reached a constant weight, powdered in an agate mortar and then sieved through a 63-µm mesh (Safahieh *et al*, 2011). Approximately 1g of the sediment samples from each station was digested with 2 ml of HNO_3 and 6 ml of HCL (Merck, Darmstadt, Germany). After digestion, the samples were cooled at the laboratory temperature and made to certain volume using double distil water.

A GBC (Savant AA∑, Australia) Flame atomic absorption spectrometer was employed to determine the metals' concentrations in the samples. All the studied metals were determined with air-acetylen flame. All reagents used were of analytical grade. Concentrated stock solution of 1000 mg/l (merck, Germany) of each metal was diluted by double distilled water for instrument calibration. All the glassware were soaked in 10% nitric acid for 24 h and rinsed three times with double distilled water before use. Standard reference material (Dorm-2, muscle of Dogfish, National Research Council of Canada) was used to check the analysis accuracy (Safahieh *et al*, 2011). The result showed good agreement with the certified values. The recovery values were; 108%, 104%, 92% and 114% for Cd, Cu, Zn and Pb, respectively. The blanks were prepared in a similar manner without samples to avoid samples contamination. The prepared blanks were used to calibrate the instrument. They were also analyzed after every 10 analysis in order to auto zero of the AAS. Significant differences between heavy metals concentration in the samples of various stations were determined using One-Way analysis of variance (ANOVA) fallowed by Duncan post hoc test. The level of significance was set at $\alpha = 0.05$.

Results

The concentration of the heavy metals in the soft tissue samples, sediment and sea water is based on µg/g dry weight. The concentration of Cd, Cu, Zn and Pb (mean ± standard error) in the soft tissue of *Crassostrea sp.HZ* is shown in Table 1. There were significant differences between the concentration of Cu and Pb in the soft tissue among the stations. The maximum concentration of Cu (403.52 µg/g) and Pb (0.95 µg/g) were observed in station 5 and 6 respectively. In the case of sediment, there were significant differences for the concentrations of Cd, Cu, Zn and Pb (Table 2). The highest level of Cd (0.85 µg/g), Cu (141.42 µg/g), Zn (122.1 µg/g) and Pb (7.25 µg/g) was recorded in station1. According to table 3, there were significant differences for the concentrations of Cd, Cu, Zn and Pb. The maximum concentration of Cd (0.7 µg/g) and Cu (3.1 µg/g) were observed in S1, while the highest levels of Zn (23.03µg/g) and Pb (2.27 µg/g) were recorded in S5.

Table 1. Heavy metal concentrations in the soft tissue of *Crassostrea sp.HZ* (µg/g d. w) from the Musa estuary.

Stations	Cd	Cu	Zn	Pb
S1	13.82 ± 1.04	342.7 ± 39.86^{c}	94.85 ± 12.73	0.35 ± 0.06^{c}
S2	16.1 ± 1.76	207.2 ± 25.97^{a}	94.5 ± 8.32	0.4 ± 0.07^{c}
S3	12.75 ± 0.59	281.6 ± 29.15^{b}	91.57 ± 13.52	0.1 ± 0.01^{b}
S4	12.21 ± 0.83	273.3 ± 18.94^{b}	90.43 ± 7.68	0.12 ± 0.01^{b}
S5	16.9 ± 2.03	403.52 ± 38.4^{d}	97.37 ± 14.23	0.07 ± 0.00^{a}
S6	17.5 ± 2.07	332.12 ± 41.64^{c}	104.12 ± 21.89	0.95 ± 0.08^{d}

Discussion

The significant differences between metals concentration in the *C. sp.HZ*, sediment and sea water samples collected from different branches of Musa estuary indicated that, heavy metals do not originate from the same sources. The maximum

concentrations of the heavy metals in the all samples were observed in station 1, 5 and 6.Station1 and 5 are located close to petrochemical units where the petrochemical wastewaters are constantly discharged into the estuary. The highest level of Cd, Zn and Pb in S6 may be related to its location in Khor-Jafari. Khor-Jafari is one the tributaries of Musa estuary and is stretched along PETZONE (Petrochemical Special Economic Zone) up to Mashahr and Sarbandar cities (Safahieh *et al*, 2011; Mooraki *et al*, 2009). This creek is used as urban wastes receptors. Moreover, it receives substantial amount of petrochemical wastewater along its courses (Safahieh *et al*, 2011). Table 4, 5 and 6 also indicate the heavy metals level in some available standards and heavy metal concentrations from other part of the Persian Gulf and the word. Regarding to *C. sp.HZ*, the obtained values for this species have been converted into µg/g w. weight with a wet wt. dry wt. ratio of 0.3.

Table 2. Heavy metal concentrations in the sediment samples (µg/g d. w) of the Musa estuary.

Stations	Cd	Cu	Zn	Pb
S1	0.85 ± 0.01^{c}	141.42 ± 21.09^{c}	122.1 ± 18.94^{c}	7.2 ± 1.03^{d}
S2	0.15 ± 0.00^{a}	21.17 ± 2^{b}	104.52 ± 12.05^{c}	2.97 ± 0.77^{b}
S3	0.4 ± 0.01^{b}	20.2 ± 3.28^{b}	63.5 ± 11.92^{a}	5.55 ± 0.94^{c}
S4	0.4 ± 0.01^{b}	1.99 ± 0.41^{a}	62.75 ± 6.54^{a}	3 ± 0.28^{b}
S5	0.32 ± 0.05^{b}	34.57 ± 2.7^{b}	60.42 ± 8.32^{a}	2 ± 0.31^{a}
S6	0.67 ± 0.08^{c}	2.16 ± 0.36^{a}	80.6 ± 10.27^{b}	4.55

Table 3. Heavy metal concentrations in the sea water samples (µg/l) of the Musa estuary.

Stations	Cd	Cu	Zn	Pb
S1	0.7 ± 0.01^{d}	3.1 ± 0.42^{d}	10.1 ± 1.07^{a}	0.55 ± 0.01^{c}
S2	0.12 ± 0.00^{a}	1.07 ± 0.2^{b}	9.72 ± 1.66^{a}	0.57 ± 0.02^{c}
S3	0.5 ± 0.01^{c}	0.87 ± 0.52^{c}	17.92 ± 2.15^{b}	0.15 ± 0.00^{a}
S4	ND	ND	9.12 ± 1.42^{a}	0.22 ± 0.01^{b}
S5	0.4 ± 0.01^{b}	ND	23.03 ± 4.58^{c}	2.27 ± 0.01^{f}
S6	0.52 ± 0.02^{c}	0.12 ± 0.00^{a}	10.35 ± 1.94^{a}	0.97 ± 0.03^{d}

Different letters show significant differences of metal concentration between Stations.

In this area, Azimi *et al* (2010) showed the highest level of Pb and Cu in soft tissue of *Crassostrea gigas*, there was in Petrochemical quay (S1station in this study). It was 11.73 and 517.32 µg/g for Pb and Cu, respectively. Furthermore they found the highest level of Cd, had been located in 15quay. It was 15.18µg/g. As well as Safahieh *et al* (2010) showed highest level of Cu and Pb in soft tissue of *C. gigas* was found in Dock SorSoreh quay (S2 in this study). It was 554.4 and 27.7 µg/g, respectively. They demonstrated found the maximum level of Cu and Pb in sediments of Bandar Emam in Dock sorsoreh quay. It was 24.1 and 18.7 µg/g, respectively.

Table 4. Heavy metal concentration in *C. gigas* muscle (µg/g d. w) from guidelines.

Standard	Cd	Cu	Zn	Pb	References
FAO (1983)	0.5 (ppm)	30 (ppm)		0.5 (ppm)	
WHO (1996)		30(mg/kg)		0.5(mg/kg)	
Turkish Guidelines[a]	0.1 (µg/g)	20 (µg/g)		1 (µg/g)	Demirak *et al*, 2006
EC[b]	0.05 (µg/g)			0.5 (µg/g)	Safahieh *et al*, 2011
NHMRC	2 (µg /g W.W)	30(µg /g W.W)	5.5(µg/gW.W)		Safahieh *et al*, 2011

Different letters show significant differences of metal concentration between Stations. FAO (1983), WHO (1996), NHMRC= National Health Research Council (Australia), EC= European Communities.

Table 5. Heavy metal concentration in sediment (µg/g d. w) from other study area and guidelines.

Stations/Standards	Cd	Cu	Zn	Pb	References
Musa estuary	0.15-0.85	1.99-141.42	60.42-122.1	2-7.2	This study
Musa estuary	0.32-0.55	9.24-18.7		2.14-4.70	Safahieh *et al*, 2011
Persian Gulf - Bahrain	0.182	48.3	52.2	99	de Mora *et al*, 2004
Persian Gulf –Oman	0.14	6.66	11.4	0.44	de Mora *et al*, 2004
North Persian Gulf	2.89			90.47	Pourang *et al*, 2005
ROPME (1999)	1.2-2			15-30	
ISQG	0.7	18.7	124	30.2	Mooraki *et al.*, 2009
PEL(Canadian SQG)	4.2	108	271	112	Mooraki *et al.*, 2009
ERL (NOAA)	1.2	34	150	47	Mooraki *et al.*, 2009

Different letters show significant differences of metal concentration between Stations. ISQG= interim marine sediment quality guideline. PEL= probable effects levels. ERL= effects range low.

Regarding of sediment, the comparison showed that the concentration Cd, Cu and Pb in the present study was relatively similar to Safahieh *et al* (2011) findings, except for S1. The higher concentration of heavy metals in our study could be related to the location of S1 that is the nearest station to petrochemical units. The concentrations of Cd and Pb in Musa estuary were lower than those in the north part of the Persian Gulf (Pourang *et al.*, 2005) and higher than those recorded in the south parts of the Persian Gulf, except for Pb in Bahrain (de Mora *et al.*, 2004). The heavy metals with the concentration lower than the legal limits were Cd, Zn and Pb which, nonetheless, reached levels 2 or 3 times below the guideline. On the other hand, the concentration of Cu in S1exceed the legal limits of heavy metals regulated by ISQG, PEL and ERL.

Table 6. Heavy metal concentration in sea water (µg/l) from other study area and guidelines.

Stations/Standards	Cd	Cu	Zn	Pb	References
North Persian Gulf	0.44			5.38	Pourang
MPL for aquatic life	5			25	Gardiner and Mance, 1984
ANZECC	2			5	ANZECC, 1992
Southwestern Turkey	0.27	0.39	1.75	0.26	Demirak *et al*, 2006
Water quality criteria (CMC)	4.3	13	120	65	US EPA (1999).
Water quality criteria (CCC)	2.2	9	120	2.5	US EPA (1999).

The comparison showed that the concentration of Cd, Cu and Zn in edible part of this species are considerably higher than FAO (1983), WHO (1996), MAFF (Ministry of Agriculture Fisheries and Food, United Kingdom), NHMRC (National Health Research Council, Australia), and TEG (Turkish Environmental Guidelines), EC (European Communities) and Turkish Guideline.

As shown in Table 6, concentration of Cd in our study was similar to that previously reported by Pourang *et al* (2005) for water from the north Persian Gulf. On the other hand, the concentration of Pb in the north Persian Gulf was remarkably higher than that in the Musa estuary. In comparison with available standard, concentration of Cd, Cu, Zn and Pb in S1, S3, S5 and S6 were higher than those found in Southwestern Turkey. Furthermore the concentrations of the metals were below the maximum permissible level for aquatic life and lower than ANZECC, CMC and CCC.

Conclusion

This study was carried out to investigate the concentration of Cd, Cu, Zn and Pb in *C. sp.HZ* from six stations along the Musa estuary. Our results indicated that the levels of heavy metals in biota, sediment and water varied among stations. The results confirmed that the concentration of heavy metal in *C. sp.HZ* strongly affected by anthropogenic activities, particularly petrochemical activities. From a public health standpoint, metal concentrations found in the edible part of this species were relatively higher than the tolerance levels for human

consumption. The concentration of the heavy metals in sediment and water was less than maximum permissible levels proposed by different guideline, except for few cases.

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